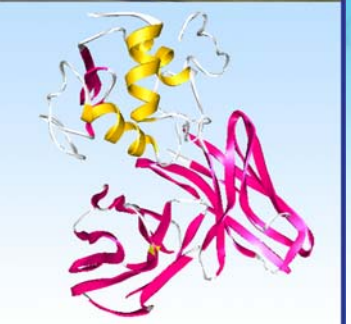


PMP Safflower Confinement at SemBioSys

Rick Keon

SemBioSys GENETICS INC.

Gene Confinement Workshop

APHIS Headquarters, Riverdale, Maryland

Sept. 13-15, 2004

SemBioSys Genetics Inc.

- Based in Calgary, Canada
- Founded in 1994 by Maurice Moloney
- 50 employees
- Safflower is used as the production platform
- Seed-based products ranging from non-transgenic formulations to pharmaceutical protein outputs
- Have conducted field trials of PMP crops for ten years

Why Safflower?

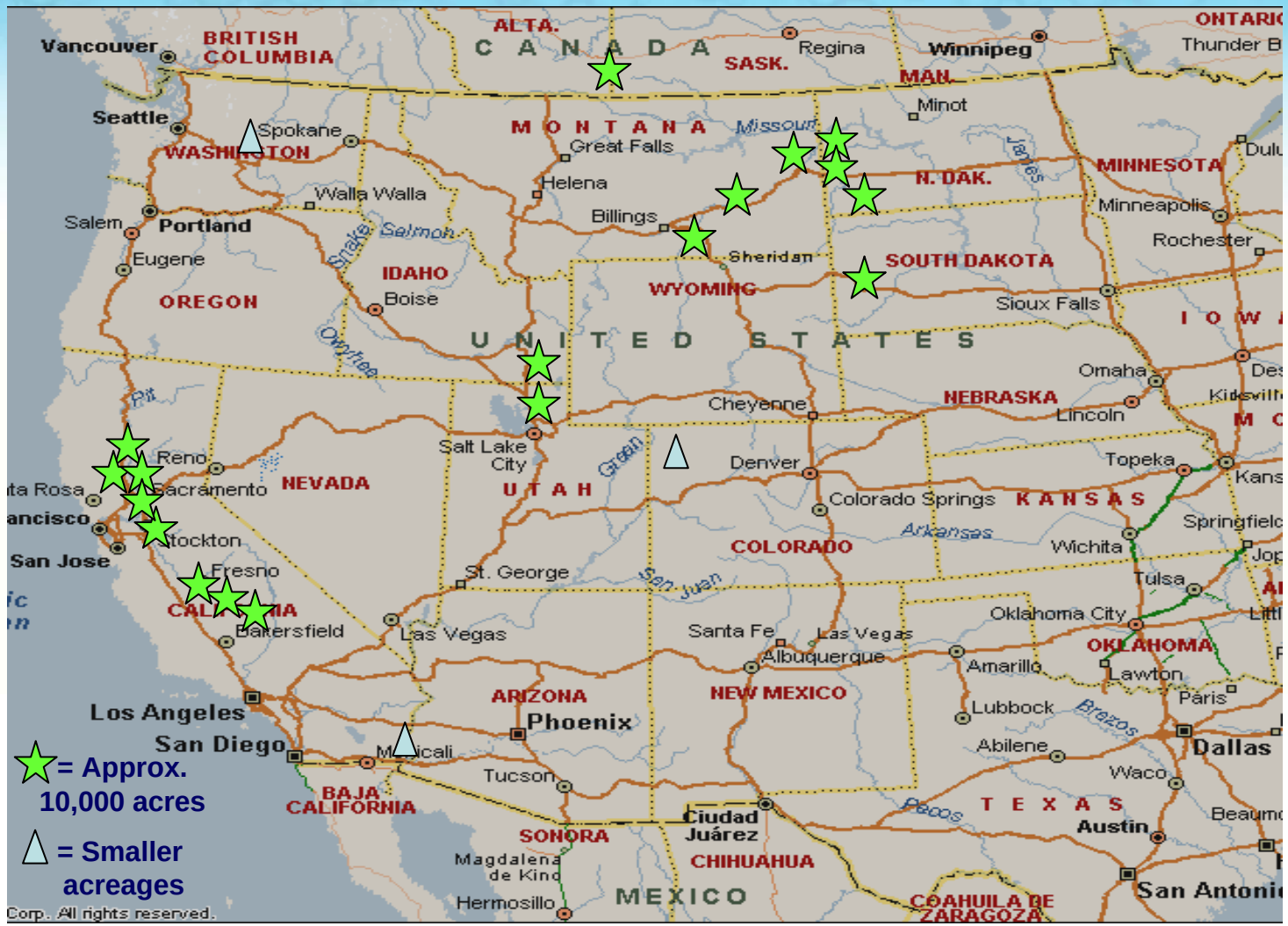
The biology of safflower makes it an excellent vehicle for PMP production.

- **Gene-flow**
  - Predominately self-pollinating (80-90%)
  - Virtually no wind transportation of pollen
  - Insects are biggest transport factors

Why Safflower?

- **Agronomy**
  - No weedy relatives found in the Americas
  - Poor volunteer
  - Low seed dormancy
  - Low vegetative dispersal
  - Low production acreages
    - <200,000 acres in N. America

North American Safflower Production



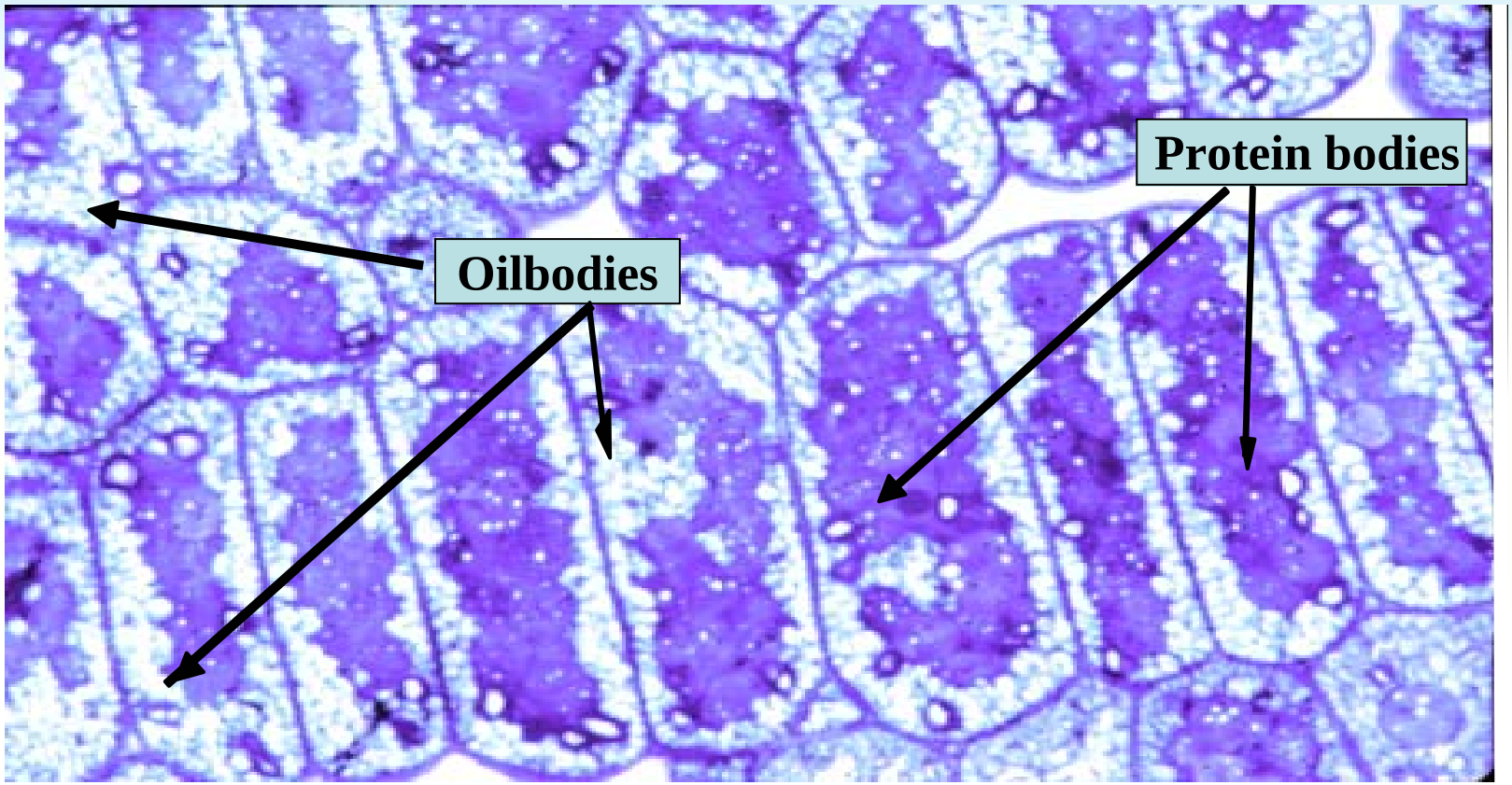
Oilseed Platform

The production of PMPs and PMIPs is targeted to the oilseeds of safflower.

- Natural protein storage organs for plants
  - high protein stability
  - can stockpile protein product
- Infrastructure exists for production and harvest
- Expression specificity
- SemBioSys' platform technology, **Stratosome™ Biologics System** offsets high purification costs

The Stratosome™ Biologics System

Typical oilseed cells contain oilbodies.

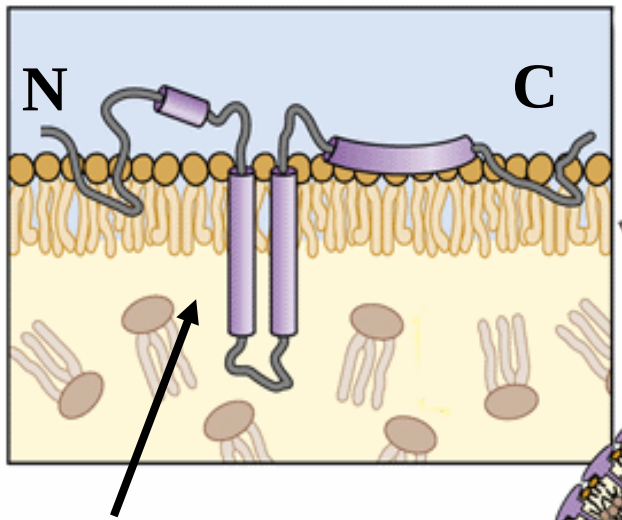


Seed Oilbodies

cytosol

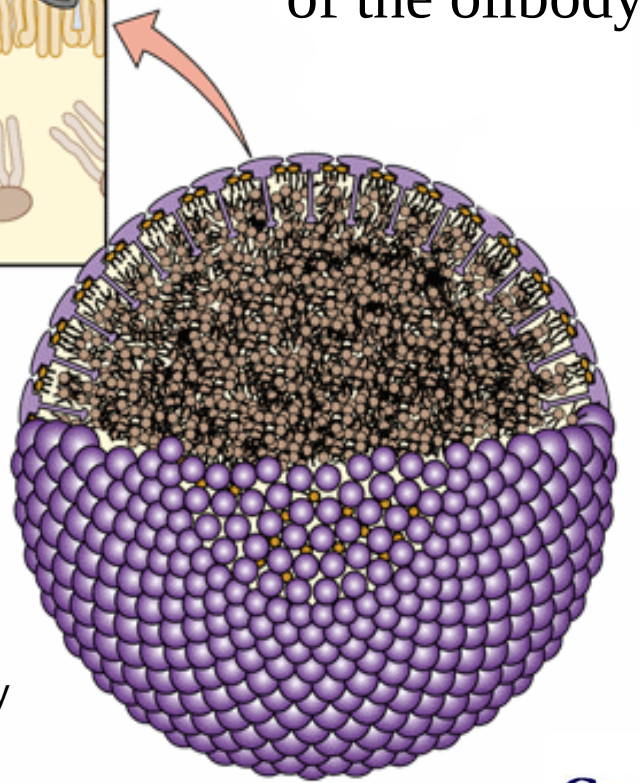
phospholipid monolayer

oil (TAG)



oleosin protein

Oleosins stud the surface of the oilbody.

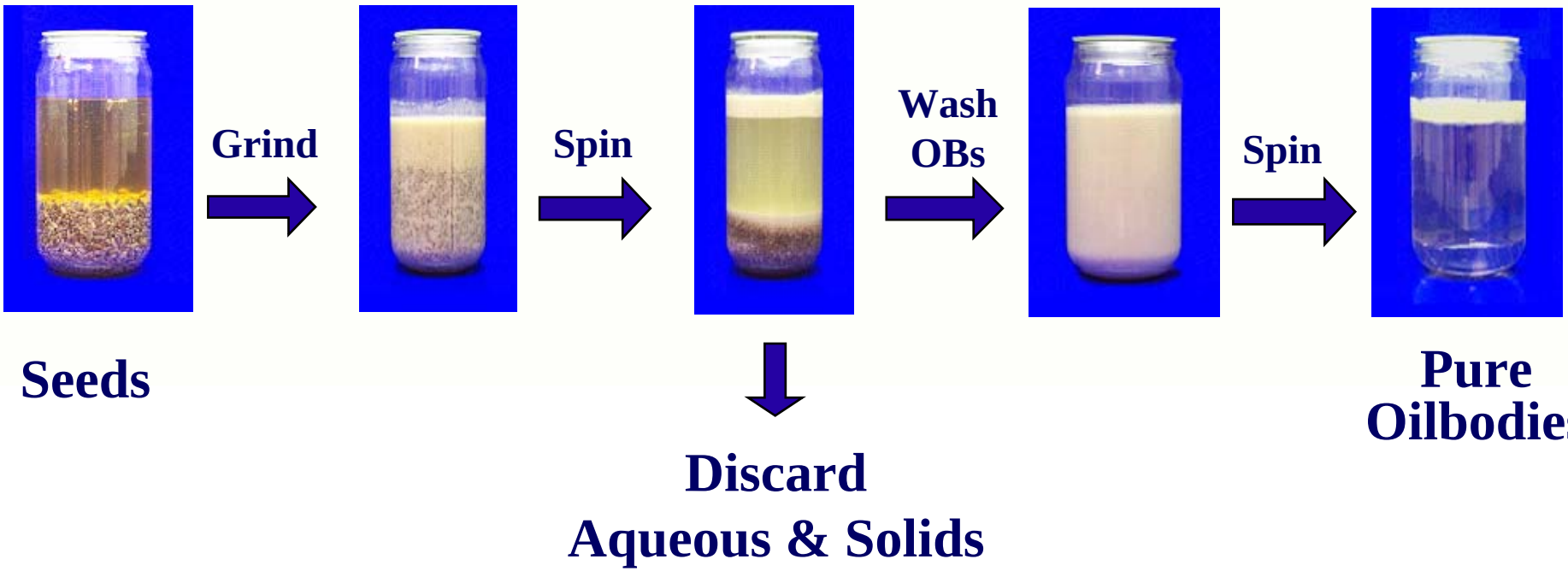


Adapted from: Biochemistry & Molecular Biology of Plants. Buchanan,Gruissem and Jones ED, 2000

SemBioSys GENETICS INC.

Purification of Oilbodies

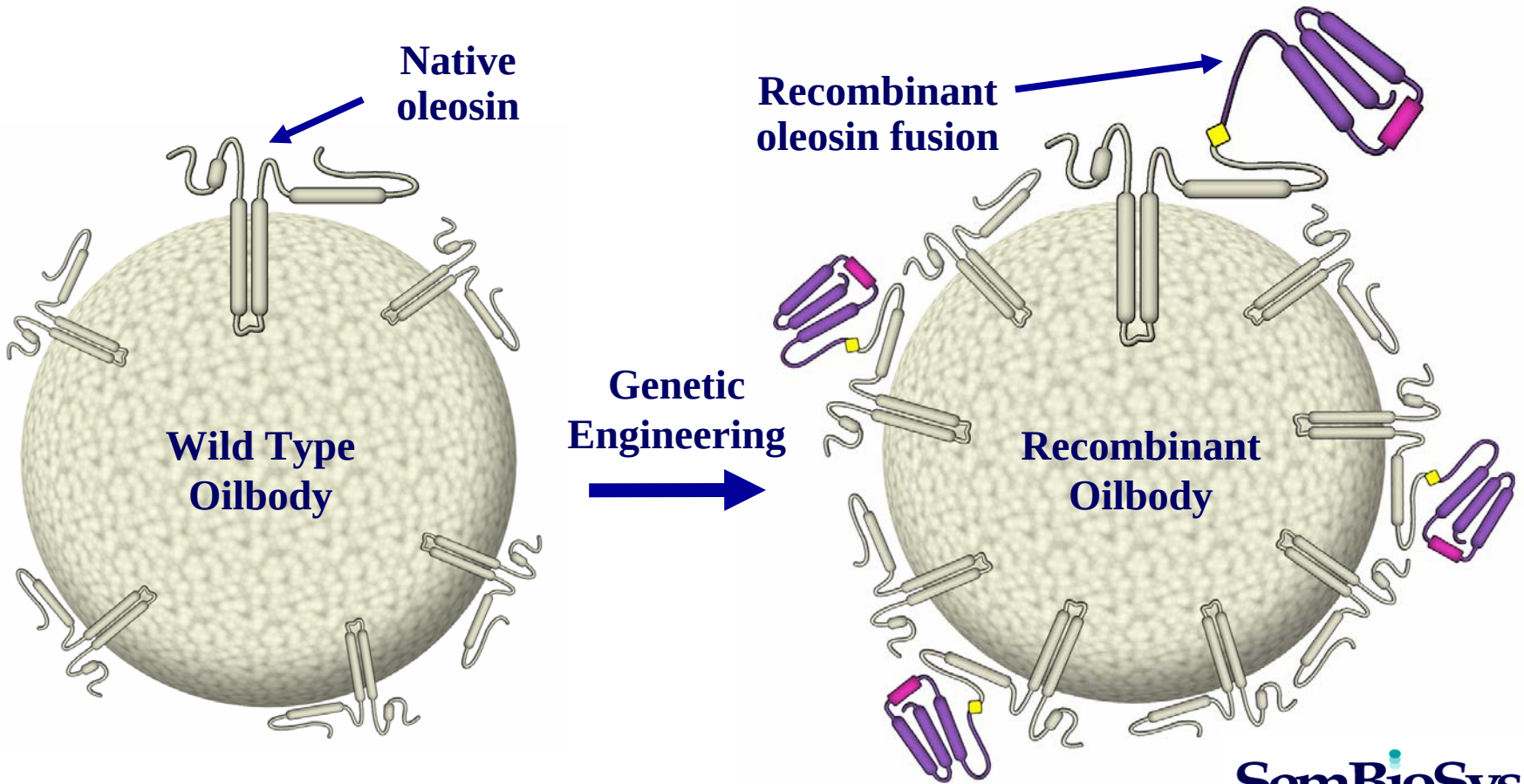
The process of flotation-centrifugation results in substantial purification of oilbody fraction.



Oilbody Preparations from Oilseed

Targeting Proteins to Oilbodies

Fuse the protein of interest to oleosin at the genetic level.

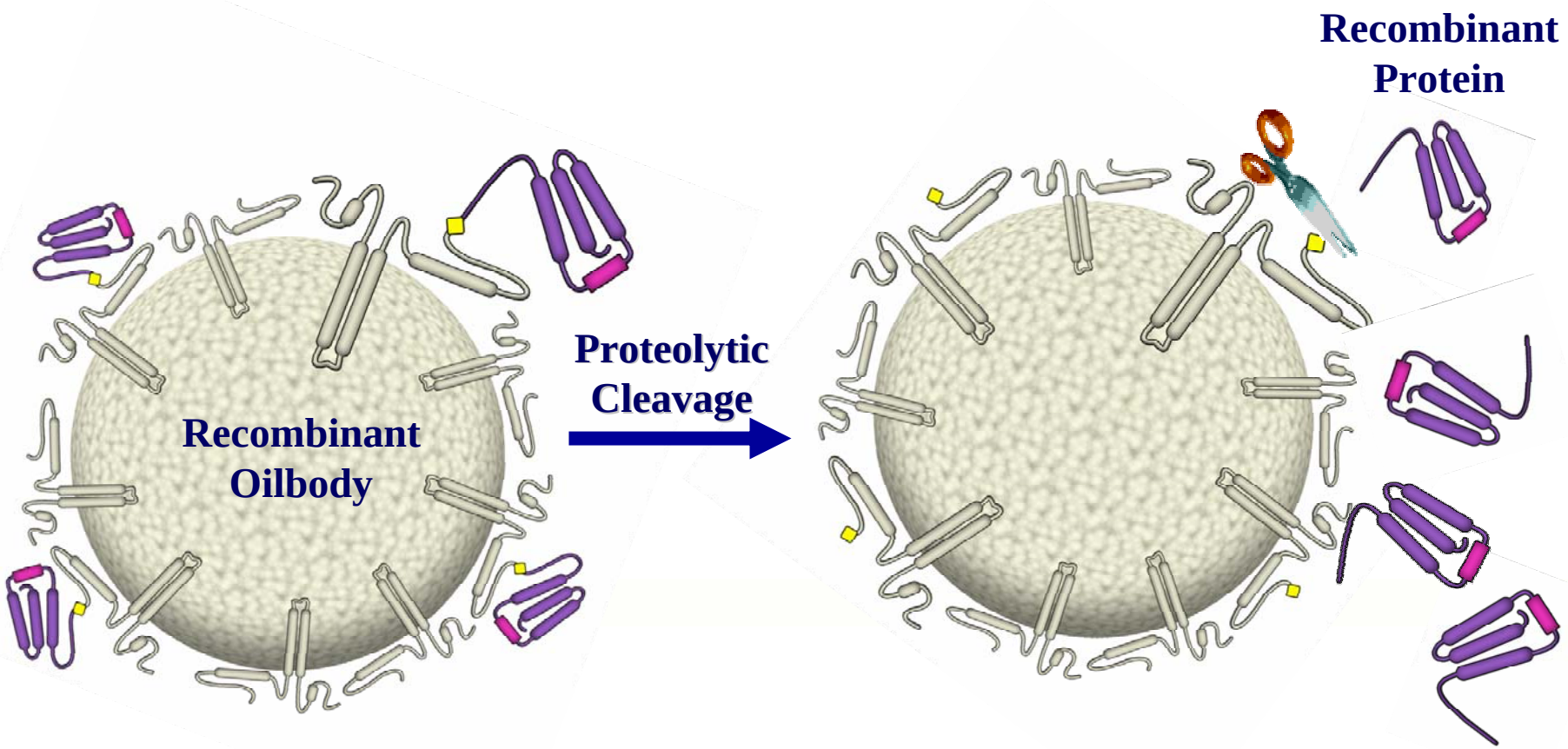


SemBioSys
GENETICS INC.

Uses of Stratosomes™

- ☞ Immobilized enzyme bioreactors
  - oilbodies form natural matrix; one-step attachment process
- Feed or food additives
  - Can boost nutritional content of seed meal
- Personal care products and topical pharmaceuticals
  - oilbodies possess great cosmetic value

Oilbodies as Purification Aids for Peptides/Proteins

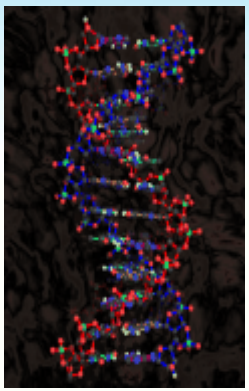


Oilbodies as Purification Aids for Peptides/Proteins

Useful in the production of pure food / feed enzymes and pharmaceuticals.

The SemBioSys Process

DNA



Tissue Culture

Transgenic safflower plant

Seed

Scale-up

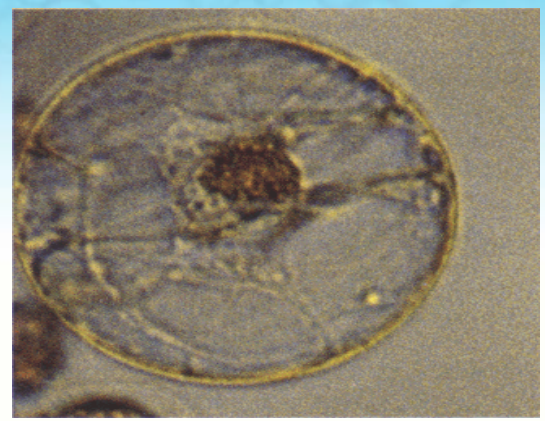
Processing

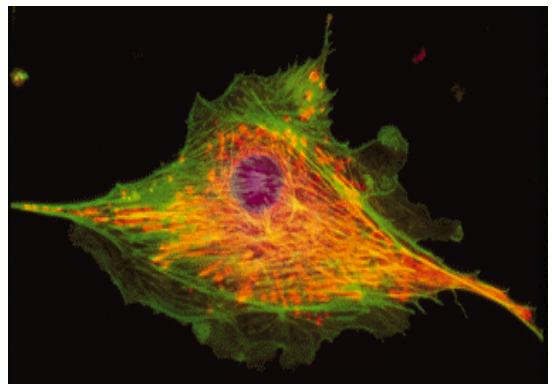
Product

Pharmaceutical Production Systems

Bacteria & fungi

- biologically contained





Animal & plant cell culture

- biologically contained

Production at SemBioSys

Industry Stewardship

Industry is committed to protecting human health and the environment.

- Rigorous compliance with confinement procedures that ensure no unintentional mixing of products with food/feed
- Sharing validated methods of analysis necessary for confirmation of confinement
- Full cooperation with agencies in audits of confinement procedures and on-site inspections
- **Development of a code of conduct**

An Industry Code of Conduct

For the PMP industry, the formation of a code has been driven by:

- A desire for stewardship and industry success
- A couple of “incidents” within the past few years
- The common use of GAPs and identity preserved systems that ensure confinement
- The recent emergence of very stringent regulations governing the planting of PMP crops
- A need for industry compliance consensus

An Industry Code of Conduct

The recently developed code brings consensus to the industry across North America.

- Compliance training programs – both country- specific and crop-specific (CropLife and Agbios)
- The Containment Analysis and Critical Control Point (CACCP) approach developed at BIO

CACCP

- Based on the food industry HACCP approach to risk assessment
- Developed by and provided to members of the BIO PMP committee as a reference for company-specific CACCP plans
- Elements covered include:
  - Plant host system characterization
  - Adherence to regulatory permit conditions
  - Personnel training
  - Contingency planning
  - Performance audits and verification
  - Safe transportation
  - Site security

CACCP

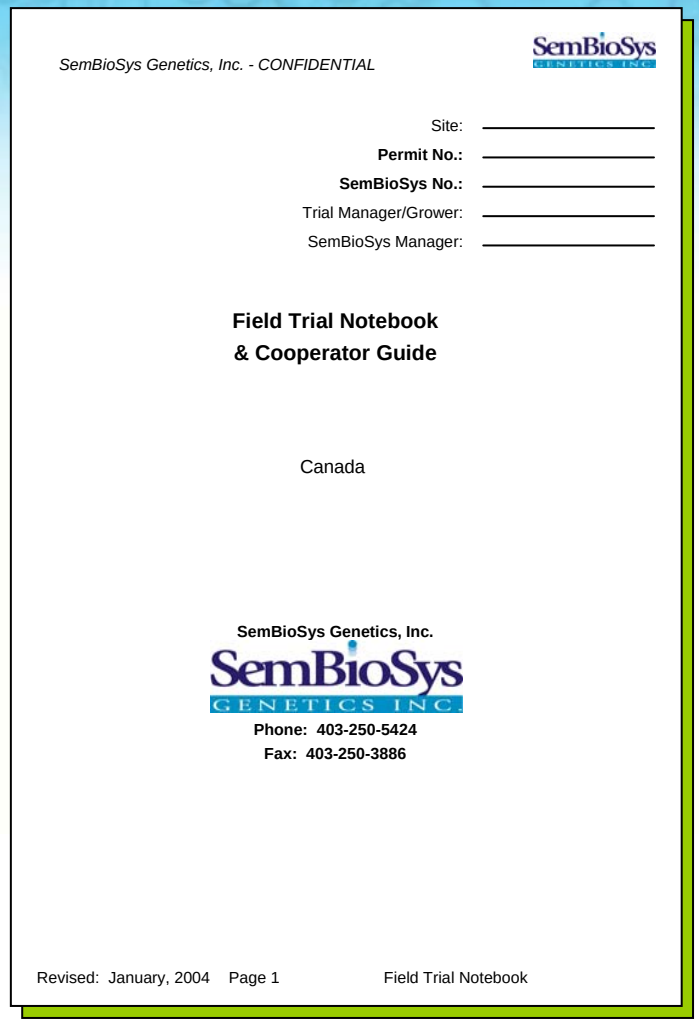
The approach covers twenty steps in the PMP production process where containment might be an issue.

- From construct development and transformation through to post-production monitoring and process auditing
- Each step goes through a rigorous assessment:
  - Analysis of Containment Concerns
  - Determine the Critical Control Points
    -Physical, Chemical, Genetic
  - Establish Critical Limits
  - Establish Monitoring Procedures
  - Establish Corrective Actions
  - Establish Verification Procedures
  - Establish Record-Keeping and Documentation Procedures

Compliance at SemBioSys

The Guide

- SOPs cover terms and conditions of:
  - CFIA Confined Field Trials for Molecular Farming crops
  - USDA Permits for Release of PMPs
- As well as CCPs in the processes



SemBioSys Genetics, Inc. - CONFIDENTIAL

SemBioSys GENETICS INC.

Site: _____

Permit No.: _____

SemBioSys No.: _____

Trial Manager/Grower: _____

SemBioSys Manager: _____

Field Trial Notebook
& Cooperator Guide

Canada

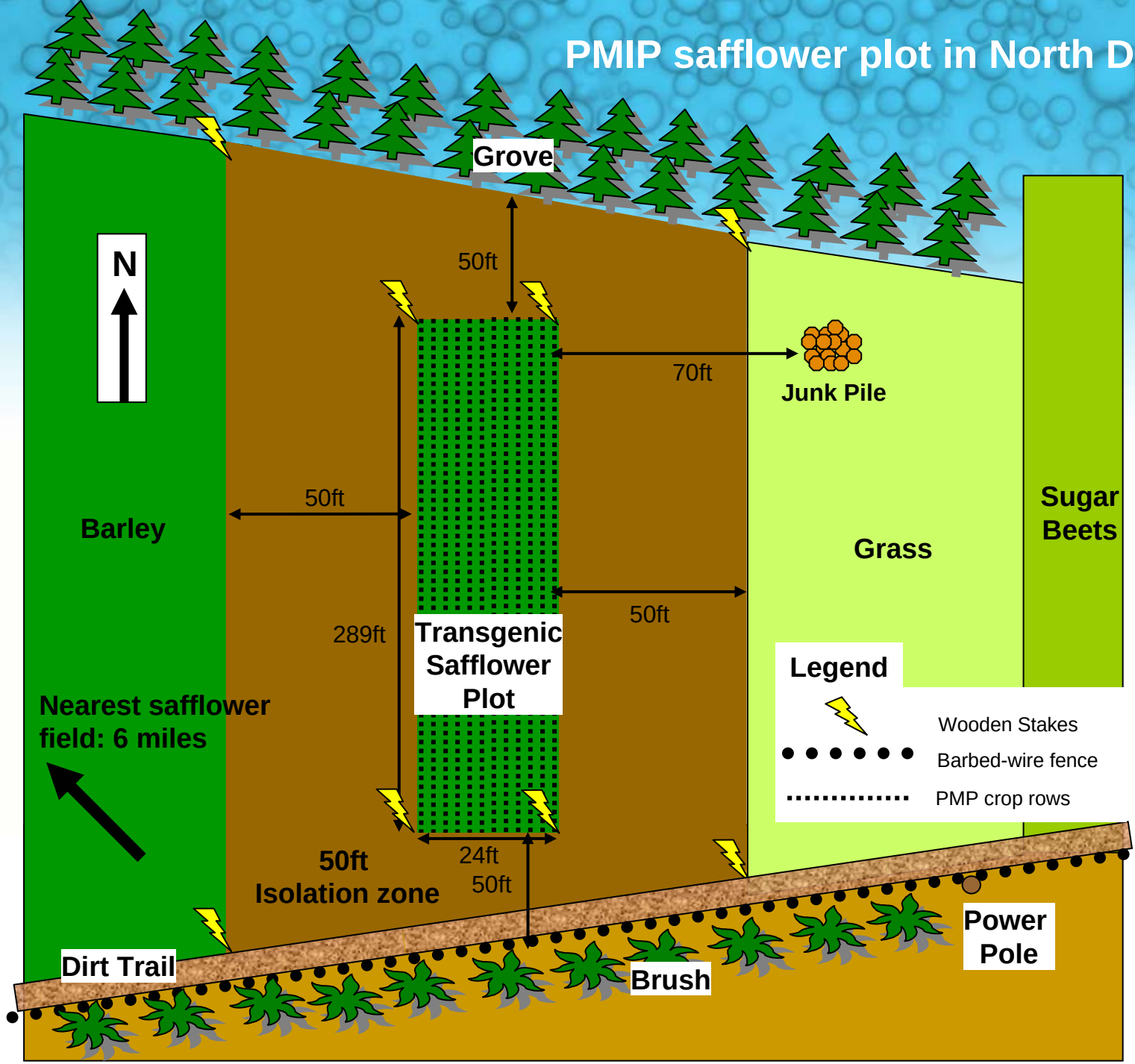
SemBioSys Genetics, Inc.

SemBioSys GENETICS INC.

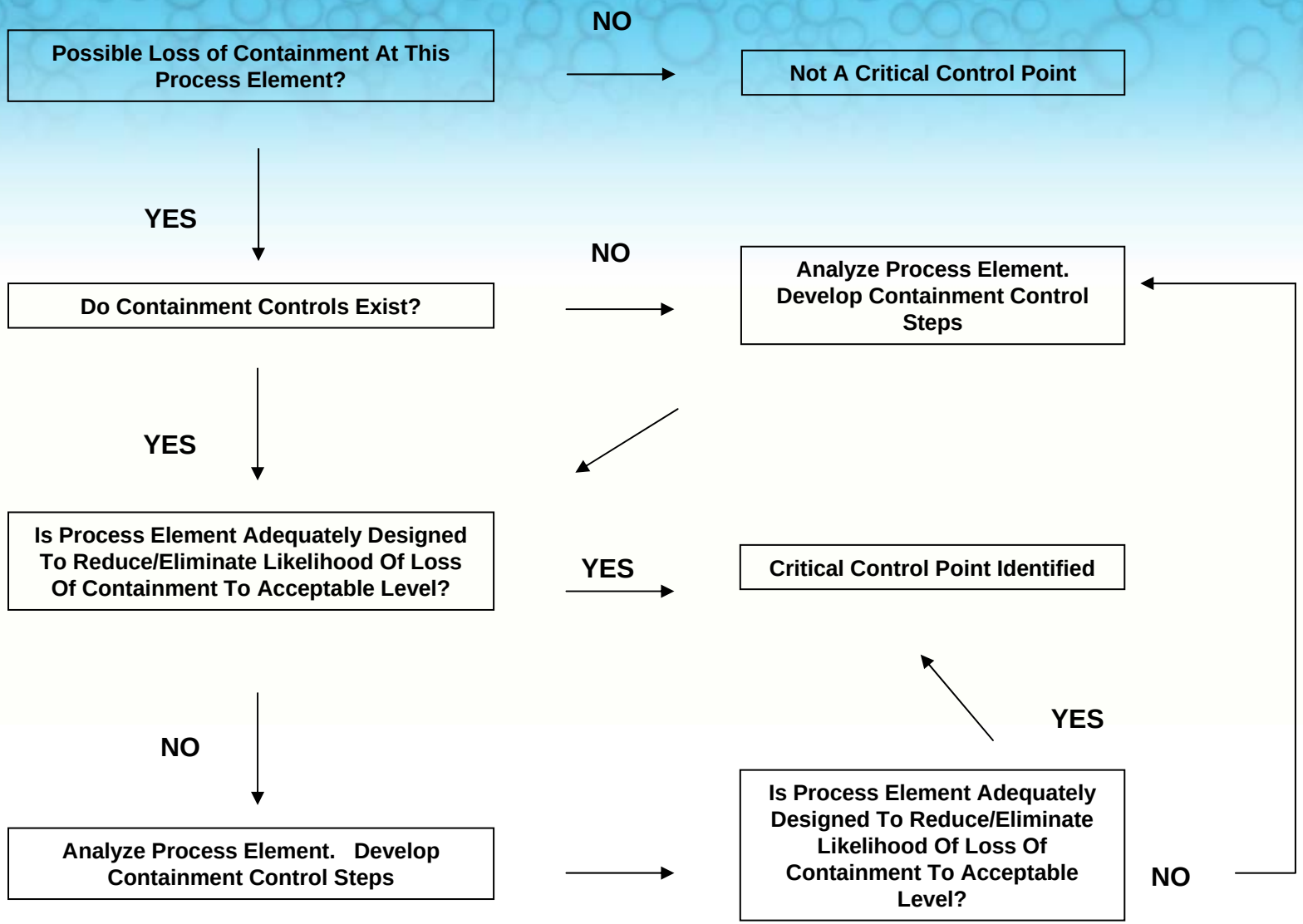
Phone: 403-250-5424
Fax: 403-250-3886

Revised: January, 2004 Page 1 Field Trial Notebook

PMIP safflower plot in North Dakota



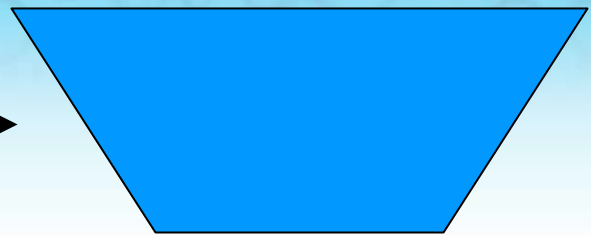
CACCP Approach



Transgenic Seed Transport and Storage

Transgenic safflower seed

Seed is double-bagged and labeled clearly



Bags placed within plastic tarps

Placed in heavy-duty molded containers or double metal boxes

Stored in secure, labeled facilities upon arrival at destination

Room for Improvement

- Furthering the cause of science-based risk assessment through agronomic research
  - Out-crossing studies with and without bees
  - Seed dormancy and overwintering studies
  - Admixture studies
  - Adaptation studies in low-use habitats
- Studies such as these lead to science-based risk-assessment of PMP growth and help to form realistic regulations when shared with authorities

In Summary…

- The PMP industry has created a code of conduct to help standardize compliance and stewardship
- This “Living” code will improve in scope and accuracy as industry continues to educate itself
- We feel that the use of crops such as safflower alongside the CACCP approach plus continued communication will help to build outside confidence in this innovative industry